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A Comparison of Management Strategies for the Federally Endangered Running Buffalo Clover (Trifolium stoloniferum) on the Blue Grass Army Depot, KY

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By

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ABSTRACT

Running buffalo clover (*Trifolium stoloniferum*) is a federally endangered plant that appears to depend on habitat disturbance, although proposed management strategies such as cattle grazing, mowing, and herbicide application have never been compared in a controlled study. We evaluate the efficacy of these techniques on the Blue Grass Army Depot (BGAD) in Madison County, KY, where one of *T. stoloniferum's* largest populations occurs. Fifty-nine patches of *T. stoloniferum* on the BGAD were treated annually between 2012 and 2014 with combinations of mowing and grass-specific herbicide. Patches of *T. stoloniferum* also were exposed to one of three types of cattle exposure (traditional dispersed grazing, enclosed grazing, and no grazing). Patches that were both mowed and sprayed with herbicide had significantly greater increases in abundance and higher survival rates than those with other treatments. Plants in any treatment group produced significantly longer and more numerous stolons than plants in control groups in the first year. Grazing status had no significant effect on abundance but ungrazed plants had significantly higher survival rates as well as significantly longer and more numerous stolons in the second year than plants in openly grazed areas. Enclosed grazing produced significantly higher increases in flower production. Although the results were sometimes inconsistent between years, they provide evidence in support of a mixed management strategy for *T. stoloniferum* that incorporates both mowing and grass-specific herbicides. The use of cattle as a management tool may hold potential, but care should be taken to regulate the duration and intensity of grazing because unrestricted grazing was more detrimental than no grazing at all.

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CHAPTER 1

INTRODUCTION

Trifolium stoloniferum (running buffalo clover) is a perennial but short-lived member of Fabaceae native to the Midwestern and Appalachian regions of the US (USFWS 2007). It produces white flowers from mid-April to June and mature seeds from May to July (Campbell et al. 1988), but it also frequently reproduces asexually through stolons that root and become independent in the Fall (Brooks 1983). Glabrous, trifoliate leaves grow basally or from nodes along stolons with distinctive stipules (Brooks 1983). *Trifolium stoloniferum* flowers are self-compatible, but require pollinator activity for fertilization (Franklin 1998). Unlike many members of its family, *T. stoloniferum* does not produce root nodules (Campbell et al. 1988) and shows no evidence of nitrogen fixation (Morris et al. 2002).

Trifolium stoloniferum is thought to have been historically abundant in the east-central US, based on several 18th century pioneer records that describe a common white-flowered clover in the Bluegrass Region of Kentucky growing at high densities (Campbell et al. 1988). These early accounts can be assumed to describe *T. stoloniferum* rather than the similar-looking and currently ubiquitous white clover (*T. repens*) because they were recorded prior to the exotic *T. repens'* colonization of woodlands in the region (Campbell et al. 1988). *Trifolium stoloniferum's* range included Arkansas, Illinois, Indiana, Kansas, Kentucky, Missouri, Ohio, and West Virginia, and tended to occur in partially shaded woodlands near rivers and streams (Brooks 1983) and associated with limestone geology (USFWS 2007).

Trifolium stoloniferum populations experienced a severe decline in the 19th century with only five known populations remaining by 1900 (Brooks 1983). In 1940 the plant was last seen at a site in West Virginia and was thought to be extinct 40 years later (Bartgis 1985). In 1983, it was rediscovered in two small populations in West Virginia (Bartgis 1985). The US Fish and Wildlife Service listed the plant as “endangered” in 1987 (USFWS 2007); in subsequent years additional populations were found in West Virginia, Ohio, Indiana, Missouri, and Kentucky, with most occurring in the Bluegrass and Appalachian regions (USFWS 2007). As of 2011, there were 116 known populations of *T. stoloniferum* and the criteria for down- listing the species to “threatened” had been met (USFWS 2011).

The reasons for the decline of *T. stoloniferum* are unknown, but researchers speculate that it depended on herds of grazing bison (*Bison bison*) to maintain its habitat (Bartgis 1985, Campbell et al. 1988). Historic pioneer accounts of *T. stoloniferum* (Nourse 1775, as cited by Campbell et al. 1988) and extant populations (Bartgis 1985) are often closely linked with areas such as mineral licks where bison once congregated; the name “running buffalo clover” probably refers to this association (Campbell et al. 1988). Recently discovered populations tend to be found in disturbed habitats such as trail sides, lawns, and stream banks (USFWS 2007). *Trifolium stoloniferum* appears to be easily outcompeted by other plants and may have depended on the grazing and trampling of bison to provide the disturbance that maintained its habitat (Campbell et al. 1988). When bison were hunted to extinction in Kentucky in the eighteenth century, most populations of *T. stoloniferum* were presumably lost to plant competition and habitat succession (Bartgis 1985). *Trifolium stoloniferum* may have also relied on bison as a source of fertilizer, seed scarification, and dispersal (Campbell et al. 1988).

Little is known about *T. stoloniferum’*s habitat requirements, and various strategies have been proposed as replacements for the historic disturbance regimens that once maintained its populations (USFWS 2007). In West Virginia, researchers suggest the disturbance from intermittent uneven-aged logging has helped to increase stem density of *T. stoloniferum* along skid roads (Burkhart et al. 2013). Managers of a county park in Ohio attribute an increase from 100 to over 2000 *T. stoloniferum* individuals over thirteen years to an intensive mowing schedule (twice before flower growth and once after fruit maturity (Becus and Klein 2002). Grazing and trampling by cattle may function as a surrogate for bison disturbance and provide seed dispersal and fertilization services. Unlike most other clover species, *T. stoloniferum* is not a host to nitrogen-fixing rhizobia (Morris et al. 2002) and so may benefit from the nitrogen fertilizer contained in bison or cattle dung (Campbell et al. 1988). *Trifolium stoloniferum* seeds have very low germination rates without scarification (Campbell et al. 1988) and it is possible (though untested) that digestion by bison or cattle provide scarification as well as a means of dispersal. Ford et al. (2003) tested the germination rates of seeds digested by white-tailed deer (*Odocoileus virginianus*), but found survival and germination rates to be low. *Trifolium stoloniferum* has been observed to be a poor competitor with other plants and might be limited by several exotic species (Campbell et al. 1988, USFWS 2007). Controlling invasive plants may be an important strategy in maintaining *T. stoloniferum* populations (USFWS 2007).

The greatest density of *T. stoloniferum* populations in the United States occurs on the Blue Grass Army Depot (BGAD) in Madison County, KY (USFWS 2011). *Trifolium stoloniferum* was discovered at the BGAD in 1992. Between 2003 and 2014, Eastern Kentucky University conducted surveys on the BGAD nearly every year and discovered *T. stoloniferum* populations can fluctuate dramatically within a few years (Dart-Padover et al. in press, USFWS 2007). The abundance of *T. stoloniferum* dropped from its greatest recorded number to its lowest in four years (from 9404 individuals in 2006 to 2367 in 2010, Dart-Padover et al. in press). While abundances rise and fall (and have been increasing from 2012 to 2014, Dart-Padover et al. in press), the number of known patches at the BGAD has steadily declined. At its peak the facility contained 134 patches, of which over half are now extirpated, with only 57 remaining in 2014.

The continued presence of *T. stoloniferum* on the BGAD, while it has vanished from the surrounding landscape, is often attributed to differences in land use history. The BGAD avoided the intensive agricultural pressures that impacted most of the Bluegrass region when the BGAD facilities were built in the early 1940's and nearly all patches occur on forested stream terraces that remained undeveloped by the Army (Fields and White 1996). Since the early 1950's, cattle have been allowed to graze on the BGAD in large numbers (approximately 6000 head), although the land made available for grazing and the number of cattle were reduced by half in the mid 1990's soon after *T. stoloniferum* was discovered there (White et al. 1999). It is unknown whether grazing was beneficial or detrimental to *T. stoloniferum* populations.

In the BGAD populations as well as in populations throughout Kentucky, Ohio, West Virginia, and Illinois, there is much uncertainty regarding the management and restoration process for *T. stoloniferum.* While there is near unanimous agreement that *T. stoloniferum* requires some form of disturbance to persist (Campbell et al. 1988, Homoya et al. 1989, Madarish and Schuler 2002, USFWS 2007, Burkhart et al. 2013) there is little more than uncontrolled, anecdotal data to suggest specific management strategies, especially in the Bluegrass Region (USFWS 2007). The objective of this study was to compare the effectiveness of three potentially useful *T. stoloniferum* management techniques (mowing, grazing, and herbicide application) after three applications over two years to populations on the Blue Grass Army Depot.

CHAPTER 2

METHODS

Study Area

The BGAD is a U.S. Army weapons storage facility in Madison County, KY (N 37.682054, W -84.22122). The site is located within the Outer Bluegrass section of the Interior Low Plateau physiographic province (Jones 2005), a province with a climate characterized as temperate, humid, and continental. The BGAD contains 5865 ha of gently rolling landscape consisting of 3 major vegetation types: pasture (74%), upland forest (14%), and bottomland forest (12%, Watt 2011). *Trifolium stoloniferum* patches on the BGAD are located in scattered riparian wooded areas that have received little direct human disturbance (Figure 1[1]). These sites tend to be associated with creek banks which receive sunlight filtered by the fairly open canopy. A large portion of patches occur in conjunction with high densities of coralberry (*Symphoricarpus orbiculatus*) and Japanese stiltgrass (*Microstegium vimineum*, Brown and Goode 2010).

Beginning in the early 1950's, almost all of the BGAD has been leased to farmers for grazing (Fields and White 1996). After the discovery of *T. stoloniferum,* the Army restricted the intensity and area of grazing by 50% (previously 6000 head of cattle, Watt 2011). Cattle were excluded from large areas on the west side of the facility in the late 1990's, while the rest of the BGAD, including much of its perimeter area, continued to support a substantial herd (1550 head counted in 2006). One smaller, highly restricted area that contains several *T. stoloniferum* patches has been the site of a multi-year cattle enclosure trial. Here cows are fenced in for several weeks each year to recreate a "flash grazing" regime similar to what bison may have

[1] All tables and figures are found in Appendix A and Appendix B, respectively

once provided while migrating (Tom Edwards, Biologist, Kentucky Department of Fish and Wildlife Resources, 2012, personal communication).

Terminology

This study frequently refers to groups of *T. stoloniferum* as "patches", which are defined by the Kentucky State Nature Preserve Commission as "one or more clustered running buffalo clover plants at least 7.5 meters (approximately 25 feet) from any other running buffalo clover plant" (Bloom et al. 1995), although over time some of the patches have grown closer to each other. This is distinct from and on a finer scale than the "populations" that the U.S. Fish and Wildlife Service uses to group *T. stoloniferum* on the BGAD. The populations are based on watershed location and other heuristic criteria (USFWS 2007), thus populations contain several patches.

Since *T. stoloniferum* is highly clonal and stoloniferous (USFWS 2007), it can be difficult to distinguish individual plants. In this study, it was not feasible to differentiate genets (and it is likely that many plants within a patch were genetically identical to each other), so I defined individuals as single ramets. As recommended by the USFWS (2007), I considered a ramet as a rooted crown (the central fan-shaped cluster of leaves) as well as any leaves or flowers connected to it by live stolons. Each rooted crown frequently grows stolons that may take root as far as 90cm from the main stem of the plant; these are considered a single individual as long as the stolon remains green and connected between the mother and daughter plant (USFWS 2007). The stolons usually begin to senesce and break apart in Summer-Fall (USFWS 2007), after the field work was done for this study.

Study Design

This experiment incorporated 59 *T. stoloniferum* patches scattered across the BGAD (Figure 1), comprising all known extant patches in the facility. Each patch contained live *T. stoloniferum* at the start of the project in 2012. The patches had been demarcated during previous surveys and have rectangular boundaries, marked with PVC stakes, that include all *T. stoloniferum* in the immediate vicinity. One tree near each patch was painted and marked with a numbered tag for use in locating patches in later years, and the GPS coordinates of each tagged tree were recorded. Because the distribution of plants within each patch moved slightly from year to year, I searched for *T. stoloniferum* at least 2 m beyond the perimeter of each patch and expanded patch boundaries as necessary.

Trifolium stoloniferum patches on the BGAD occur in one of three grazing situations: in areas where cattle were excluded entirely (hereafter referred to as “not grazed” or “ungrazed”; 28 patches), in a large area in which approximately 1550 head of cattle roamed freely (referred to as “open grazing”; 13 patches), and in a smaller fenced area in which a small herd of several dozen cattle (exact number varied and was unknown) was enclosed for several weeks each spring (referred to as “enclosed grazing”; 18 patches). Each patch was assigned one of four experimental treatments: mowing, grass-specific herbicide, mowing and grass-specific herbicide in combination, and control (no treatment). The patches were stratified by patch size and grazing status, and then treatments were randomly allocated to all 59 patches (Table 1). An exception was made for the nine patches which contained more than 120 individuals in 2012. Because of the conservation importance of these patches, they were all placed in the control group to avoid the risk of inadvertently harming them with treatments. Each patch was surveyed and treated three times, between May and July of 2012, 2013, and 2014. My results do not include responses following the 2014 treatments. Total precipitation in Madison County in the year prior to each survey in 2012, 2013, and 2014 was 117.3, 105.4, and 139.3 cm, respectively; average temperatures were 14.1°, 12.5°, and 11.8° C, respectively (Kentucky Mesonet 2015) , which were reasonably similar to the long-term (1981-2010) average precipitation of 114.7 cm, and temperature of 13.1° C.

Data Collection

Patch Responses

Each year, before performing manipulations, I surveyed all known patches while *T. stoloniferum* was flowering to determine that year's population characteristics. Surveys began between May 7- 15th and were finished between June 20-July 8. I counted each rooted crown and inflorescence. *Trifolium stoloniferum* abundance was represented by the number of rooted crowns in each patch. Surveys were timed to coincide with the onset of blooming for maximum flower visibility, but any green immature inflorescence or brown fruiting infructescence was included in the inflorescence count. Rarely, a peduncle had two inflorescences instead of one, in which case, both were counted.

To make the *T. stoloniferum* survey process as consistent and efficient as possible, I marked each rooted crown in a patch with a pin flag while being careful not to trample the plants and checking to ensure no marked plants were attached to each other by stolons. During this process, I counted all inflorescences. After marking every rooted crown present, the pin flags were removed and counted to obtain the abundance for a patch.

Individual Plant Responses

In order to understand how individual *T. stoloniferum* plants responded to treatments over time, I recorded additional measurements on a subsample of plants at each patch following abundance counts. Beginning from the corner of the patch nearest to the tagged tree and moving toward the center of the patch, I marked several *T. stoloniferum* individuals with a numbered aluminum tag to enable tracking in subsequent years. For each tagged plant, I measured the number of all stolons, inflorescences, and crown stems, and recorded the length of each stolon. When these subsamples were established in 2012, I tagged and measured a maximum of 50 rooted crowns per patch. In patches with fewer than 50 plants, every individual was tagged and measured. In subsequent years (2013 and 2014), all tagged plants were measured again, and any new plants within the outer boundaries of those labelled plants were tagged and measured. Midway through the second sampling season (2013), it became clear that most patches were experiencing substantial abundance increases. Measuring each plant in the previously sampled area would require an increase in time and effort that would make sampling all patches impractical (for example, an area of one patch that had 50 rooted crowns in 2012 had 143 rooted crowns in 2013). In cases with more than 50 individuals in an area to be subsampled, a 1 m^2 quadrat was placed in the area with the greatest density of previously tagged plants in order to limit the number of newly tagged plants. When a quadrat was used, its boundaries were marked with flags and used again the next year. Any individuals that were previously tagged outside of the quadrat continued to be measured every year, but no new individuals were tagged and measured outside the quadrat area.

Treatments

Manipulations began each year as soon as *T. stoloniferum* seeds matured and the surveys were complete, i.e., between June 14 and July 3. Patches with the mowing or mowing+herbicide treatment were cut to a height of approximately 7.5 cm using a gas-powered string trimmer. A one meter buffer around each patch perimeter was also mowed. Woody shrubs were cut with hand trimmers (and in some cases a weed eater with metal blade) and removed from the patch.

Fusilade II, a postemergent grass specific herbicide with active ingredient Fluazifop-P-butyl (Syngenta Crop Protection, Inc., Greensboro, NC), was mixed with Activator 90 non-ionic surfactant (Loveland Products, Inc.) and applied to *T. stoloniferum* patches on the BGAD assigned the herbicide treatment at a rate of 81 ml per 100 m^2 with a backpack sprayer between June and July. A one meter buffer around each *T. stoloniferum* patch was also sprayed to prevent the immediate recolonization of surrounding grasses. Patches with the mow+herbicide treatment were mowed first and clumps of cut vegetation were removed prior to spraying with herbicide. All treatments were finished by July 16 each year. The active ingredient of the herbicide is transported throughout the plant and interferes with lipid synthesis in monocots, preventing cell membranes from forming (Walker et al. 1988). The manufacturer suggests Fusilade II will not harm broadleaf plants. To gauge its effect on *T. stoloniferum* prior to treatment, I applied Fusilade II to six clover plants in a restoration population outside of the BGAD. One week later small brown spots were observed where the herbicide had directly contacted leaves, but the damage appeared to be superficial and localized because all affected plants continued to grow.

Statistical Analysis

Data were collected in two forms and were analyzed separately in SPSS (version 20, IBM 2011): (1) Overall patch responses (percent changes in abundance and numbers of inflorescences per plant), and (2) individual plant responses derived from the patch subsamples (survival and recruitment data, number and length of stolons per plant). Each year, or change over one or two years, of the patch-level responses were analyzed with a univariate ANOVA with treatment, grazing status, and the interaction between treatment and grazing as independent factors. If any of the analyses failed to fulfill the assumption of homogeneity of variance (with Levene's test producing a p-value < 0.05), I removed any data that were clear outliers (had a studentized residual of less than -3 or greater than 3) and conducted the analysis again. With each analysis, post-hoc pairwise comparisons for both treatment and grazing factors were performed with Tukey's HSD test.

I evaluated *T. stoloniferum's* abundance responses as the percent change in rooted crowns (change in rooted crowns/initial number of rooted crowns) rather than simply the change in number of rooted crowns, because the relative change in abundance avoids bias that might result from the uneven distribution of treatments among the most abundant patches. The *T. stoloniferum* survival rates for each patch were estimated as the percent of previously tagged plants that were found alive in subsequent years. Average patch survival rates between years were analyzed using a univariate ANOVA with treatment and grazing as independent factors. Using the subsample of tagged individuals for each patch, I estimated the recruitment rates, which consist of the number of new plants within a subsample divided by the number of tagged plants alive in the previous year within the same subsampled area. Average patch recruitment rates were also compared with a univariate ANOVA with treatment and grazing as independent factors.

Each year of the individual plant data was analyzed separately, with each measurement for each year serving as the response variable in a generalized linear model using a negative binomial distribution with a log link, and treatment and grazing status as main factors and the patch number as a between subjects factor. I originally planned to analyze responses with a repeated measures ANOVA, but in all cases Mauchly's Test of Sphericity indicated that the assumption of sphericity was violated and so years (or changes between years) were analyzed separately for each response variable.

CHAPTER 3

RESULTS

Abundance

The total abundance of *T. stoloniferum* across all of the BGAD increased 100.4% over two years, with individuals increasing 55.6% in 2012-2013 and 28.8% in 2013-2014 (Figure 2). While 86% of patches increased or did not change in abundance over the two years, two patches had only a single plant present in 2012 and were both were extirpated by 2013. These were excluded from all patch-level analyses.

Treatments had a significant effect on the percent change in *T. stoloniferum* abundance on the BGAD between 2012 and 2014 ($F_{3, 43} = 11.503$, $p < 0.001$; Figure 3), however the effect of grazing was not significant ($F_{2, 43} = 0.35$, $p = 0.707$; Figure 4). A single outlier patch, with very high abundance, was removed from the analysis because its inclusion was leading to violations of the ANOVA assumption of equal variances. The mow+herbicide treatment produced a significantly greater increase in abundance than the herbicide ($q = 3.09$, $p = 0.018$) and control treatments ($q = 3.88$, $p = 0.002$). Mow+herbicide did not differ from mow ($q = 2.20$, $p = 0.140$) and none of the other three treatments were significantly different from one another ($q < 1.50$ and $p > 0.451$ in all three cases). There was a significant interaction between treatment and grazing ($F_{6, 43} = 6.708$, $p < 0.001$) in which the openly grazed patches with the mow+herbicide treatment had a greater increase in abundance than any other combination of treatment and grazing (Figure 5). However, it should be noted that this group contained only two patches.

Changes in *T. stoloniferum* abundance on the BGAD over single years followed similar patterns, with treatment generating significant differences in abundance between both 2012-2013 and 2013-2014 (2012-2013: $F_{3, 44} = 5.317$, $p = 0.003$; 2013-2014: $F_{3, 45} = 4.685$, $p = 0.006$;

Figure 6). Post-hoc tests indicated that mowing alone was associated with a greater increase in abundance than the control group during the first year (q = 3.35, p = 0.009), and patches that received mowing+herbicide had a greater increase in abundance than the mowed only patches during the second year (q = 2.92, p = 0.027; Figure 7). As with the two year span, grazing status produced no significant difference over single years (2012-2013: $F_{2, 44}$ = 0.45, p = 0.584; 2013-2014: $F_{2, 44}$ = 0.242, p = 0.786; Figure 7). There was no significant interaction between treatment and grazing status for abundance in either year (2012-2013: $F_{6, 44}$ = 2.026, p = 0.082; 2013-2014: $F_{6, 45}$ = 2.232, p = 0.057).

Inflorescences

The total number of *T. stoloniferum* inflorescences counted in the BGAD more than doubled between 2012 and 2013, from 924 to 2054. The number of inflorescences declined slightly between 2013 and 2014. Reflecting this trend, the average (± SE) number of inflorescences produced by each plant rose from 0.22 (± 0.02) in 2012 to 0.37 (± 0.02) in 2013, before dropping back to 0.25 (± 0.01) in 2014.

The mean change in the number of *T. stoloniferum* inflorescences per plant on the BGAD between 2012 and 2014 did not differ significantly by treatment ($F_{3, 44}$ = 1.043, p = 0.383; Figure 8) but it was significantly affected by grazing status ($F_{2, 44}$ = 5.866, p = 0.006). Post-hoc tests indicated that the patches within the enclosed grazing areas had a significantly greater increase in inflorescences per plant than either patches in openly grazed (q = 3.00, p = 0.012) or ungrazed areas (q = 2.74, p = 0.024; Figure 9). There was no significant interaction between treatment and grazing status in 2012-2014 ($F_{6, 44}$ = 0.510, p = 0.797). Treatments again had no significant effect on inflorescence change over either of the single years 2012-2013 or 2013-2014 ($F_{3, 44}$ = 0.953, p = 0.423; $F_{3, 43}$ = 0.996, p = 0.404; Figure 10). Grazing had a significant effect on the change in number of inflorescences per plant during the single year between 2012 and 2013 ($F_{2, 44}$ = 3.613, p = 0.035), though not between 2013 and 2014 ($F_{2, 43}$ = 0.136, p = 0.873). During 2012-2013, enclosed grazed plants again had a significantly greater increase in inflorescences than not grazed plants (q = 2.51, p = 0.041; Figure 11). There was no significant interaction between treatment and grazing status in either year (2012-13: $F_{6, 44}$ = 1.502, p = 0.200; 2013-14: $F_{6, 43}$ = 1.479, p = 0.207).

Survival

The overall average survival rate of *T. stoloniferum* across the BGAD differed by year (0.66 in 2012-2013 and 0.59 in 2013-2014). The percentage of *T. stoloniferum* plants that were first tagged in 2012 and survived two years until the 2014 survey was 40%. Average survival rates per patch differed significantly by both their treatment and grazing status. Treatment had a significant effect on survival rates between 2012 and 2014 ($F_{3, 44}$ = 3.105, p = 0.036). Although the effect was non-significant after adjustments for multiple comparisons, the patches that received both mowing and herbicide had greater survival rates (mean ± SE = 0.52 ± 0.06) than patches with only mowing, herbicide, or control treatments (mean ± SE = 0.32 ± 0.06, 0.28 ± 0.06, and 0.38 ± 0.05 respectively) between 2012 and 2014 (Figure 12). Survival rates between 2012 and 2014 differed significantly by grazing ($F_{2, 44}$ = 4.174, p = 0.022), with multiple comparisons showing ungrazed patches had significantly higher survival (0.47 ± 0.04) than the openly grazed patches (0.27 ± 0.06, q = 3.04, p = 0.011; Figure 13). Survival rates in the enclosed grazing area were not significantly different than those of either of the other grazing statues. There was no significant interaction between treatment and grazing status between 2012 and 2014 ($F_{6, 44}$ = 1.318, p = 0.269).

When comparing *T. stoloniferum* survival rates over the single years between surveys, differences among experimental groups on the BGAD were not apparent. Neither treatment nor grazing was associated with significantly different survival rates in 2012-2013 (treatment: $F_{3, 44}$ = 0.73, p = 0.539; grazing: $F_{2, 44}$ = 2.670, p = 0.08) and there was no significant interaction between the two factors ($F_{6, 44}$ = 1.025, p = 0.422). The survival data for 2013-2014 had unequal variances among groups, even after attempted transformations, and could not be analyzed. However, for 2012-2013 and 2013-2014, the same treatment (mow and herbicide) and grazing management (not grazed) had the highest mean survival rates (Figure 14 and Figure 15).

Stolons

Overall *T. stoloniferum* stolon growth on the BGAD declined each year, beginning with a mean (± SE) of 1.40 (± 0.04) stolons and a combined length of 34.58 (± 1.17) cm per plant in 2012, and dropping to a mean of 0.99 stolons (± 0.02) with a total length of 17.36 (± 0.59) cm per plant in 2014. After one year of treatment, in 2013, the average number of stolons per plant significantly differed by treatment (Wald Chi-Square_3 = 9.028, p = 0.029). Pairwise comparisons showed that individuals that received any of the three treatments had significantly more stolons per plant than the control plants (Figure 16). The average total length of stolons per plant showed a similar relationship in regards to treatment (Wald Chi-Square_3 = 8.255, p = 0.041); whereas plants that were either mowed or sprayed with herbicide had longer stolons than control plants, and those that received the mow+herbicide treatment did not differ from any other treatment group (Figure 17). Grazing status had no significant effect on either the number of stolons (Wald Chi-Square_2 = 4.995, p = 0.082), or the lengths of stolons (Wald Chi-Square_2 = 2.788, p = 0.248) in 2013 (Figure 18 and Figure 19). However, these relationships reversed after two years of treatment. In 2014 the treatments had no significant effect on numbers of stolons per plant (Wald Chi-Square_3 = 5.156, p = 0.161) or lengths of stolons per plant (Wald Chi-Square_3

= 1.837, p = 0.607), but grazing status did. In 2014, the number of stolons and the total stolon length per plant differed significantly with grazing (Wald Chi-Square$_2$ = 7.128, p = 0.028 and Wald Chi-Square$_2$ = 8.056, p = 0.018, respectively), with plants in both enclosed grazing and ungrazed areas growing more and longer stolons than openly grazed plants (Figure 18 and Figure 19).

Recruitment

Average *T. stoloniferum* recruitment rates (± SE) on the BGAD were 1.15 (± 0.11) new individuals per previously existing plant in 2012-2013 and 0.78 (± 0.08) new individuals per previously existing plant in 2013-2014. The average 2012-2013 and 2013-2014 recruitment rates did not significantly differ by either treatment ($F_{3, 43}$ = 0.087, p = 0.967 and $F_{3, 42}$ = 1.670, p = 0.188, respectively) or grazing status ($F_{2, 43}$ = 0.757, p = 0.475 and $F_{2, 42}$ = 0.033, p = 0.922, respectively; Figure 20 and Figure 21). The 2012-2013 recruitment data were transformed ($\log_{10}$ of value + 0.5) and had one outlier removed in order to meet the assumption of equal variance among groups. There was a significant interaction between treatment and grazing for 2012-2013 recruitment ($F_{6, 43}$ = 4.043, p = 0.003) in which patches within the grazing enclosure that received mowing and herbicide had greater recruitment rates than any other combination of treatment and grazing status; Figure 22). There was no such interaction affecting 2013-2014 recruitment ($F_{6, 42}$ = 0.356, p = 0.902).

CHAPTER 4

DISCUSSION

A combination of both mowing and application of grass-specific herbicide produced the strongest increases in patch abundance and *T. stoloniferum* survival rates after two years of annual treatments. Using these two methods in conjunction with one another may be the most effective way for land managers at the BGAD to preserve and grow the species' populations. The success of these techniques indicates that ground-level plant competition is an important limiting factor for *T. stoloniferum* on the BGAD, as suggested by the USFWS (2007) and others (Campbell et al. 1988, Homoya et al. 1989).

In this study mowing and applying herbicide proved to be beneficial for *T. stoloniferum* when used in combination, and one might expect that either technique alone would also be beneficial. However, this was not the case and neither *T. stoloniferum* patches with only mowing nor only herbicide had significantly different abundance increases or survival rates than untreated patches. In a previous experiment at the BGAD, White et al. (1999) found that the frequency of *T. stoloniferum* plants in patches treated with a grass-specific herbicide (Poast) actually decreased over two years. One possible explanation for these counterintuitive results is that each treatment inhibits one group of plant competitors (forbs in the case of mowing and grasses in the case of herbicide) while allowing the other to thrive. For example, if mowing only serves to enhance grass colonization, then perhaps mowing alone has no net benefit to *T. stoloniferum*. Control efforts targeting a particular class of exotic plants can sometimes assist other undesirable species (Choi and Pavlovic 1998, D'Antonio and Meyerson 2002).

The open grazing practiced across much of the BGAD was not effective in promoting *T. stoloniferum* growth; in fact, patches with no cattle access had significantly higher survival rates than open-grazed areas over the course of two years. Whether the higher survival in ungrazed areas is due to the absence of direct cattle-based herbivory, trampling, or some other effect is unknown. Plants in the enclosed grazing area had greater increases in inflorescences, and grazing only affected stolon development between 2013 and 2014, when open-grazed plants exhibited less growth. Grazing status had no effect on patch abundances, indicating that it may be less of a useful management technique than has been observed by Homoya et al. (1989). If cattle-grazing is to be used as an effective tool for *T. stoloniferum's* recovery, it may be necessary to carefully control the intensity and duration of grazing for there to be any benefit.

Despite the significant differences between *T. stoloniferum* growing under different grazing regimes on the BGAD, the effects of the grazing statuses should be considered with restraint, because they were strongly conflated with location on the BGAD and any observed effects could be due to local geography and habitat differences rather than cattle presence. For example, the ungrazed patches were generally clustered on the east side of the BGAD; whereas the openly grazed patches were located near the western perimeter. The enclosed grazing all took place in a relatively small area (Figure 1) that contained more upland patches than anywhere else. Furthermore, not all areas on the BGAD open to cattle appeared to have been actually visited by them. Several *T. stoloniferum* patches with open-grazed status showed no signs of cattle activity while patches in nearby areas exhibited signs of trampling. The highly localized and variable effects on soil compaction and competitor removal make cattle grazing difficult to evaluate on a large scale. Based on my own qualitative observations, cattle tended to use the forested riparian spaces for water access and travel corridors, but most grazing and congregating took place in the fields and woodland edges, far from *T. stoloniferum* patches. It may be that cattle simply do not have the same effect on *T. stoloniferum* that bison once did due to behavioral differences. Campbell et al. (1988) suggest that the high concentration and continual presence of cattle maintained by most ranchers is damaging to *T. stoloniferum*. Bison in the Ohio Valley moved in concentrated herds along well established paths (sometimes approximately 60m wide) between scattered salt licks and canebrakes (*Arundinaria gigantea*, Jakle 1968). While the enclosed grazing method was an attempt to replicate this periodic but intense migratory-related grazing, its success is unclear. Future research should continue to investigate cattle grazing as a management tool for *T. stoloniferum*, with a greater attempt to record, and possibly control, the animals' visits and behavior in clover patches.

With the widespread assessment that historic populations of *T. stoloniferum* depended on bison and elk (Bartgis 1985, Campbell et al. 1988, Cusick 1989, Homoya et al. 1989, USFWS 2007), managers should continue to seek to understand the species' interactions with modern mammals. The browsing of plant competitors by mammals is only one of several proposed animal-related benefits to *T. stoloniferum.* Others include the scarification and dispersal of seeds (Campbell et al. 1988, Cusick 1989), as well as fertilization (which may be important to a legume that has no *Rhizobium* symbiont). If cattle can provide increased germination and establishment of new patches, the methods used in this study would not have detected it. The value of manure as fertilizer for *T. stoloniferum* is unknown and should be studied, although Watt (2011) found no differences between soil nutrient levels (NH_4, NO_3, Zn, Mg, Ca, and P) in *T. stoloniferum* sites and randomly selected sites on the BGAD. If cattle are a poor ecological analog for bison, then maybe other herbivores are better surrogates. White-tailed deer, which are present on the BGAD in large numbers, have been proposed as dispersers of *T. stoloniferum* seeds (Cusick 1989), although *T. stoloniferum* seeds ingested by deer have very low survival and germination rates (Ford et al. 2003). Survival and germination of *T. stoloniferum* seeds passed through the intestinal tracts of bison, cattle, or other herbivores has not been investigated.

Of the several *T. stoloniferum* responses analyzed in this study, it is not evident which are most important for long-term population health. Patch abundance is clearly the most immediate indicator of a patch's future persistence, since the abundance of rooted crowns in a patch has been shown to be directly linked to its probability of extirpation (Dart-Padover et al., in press). But other patch characteristics may signify different (and potentially conflicting) aspects of *T. stoloniferum* population viability. For example, higher survival rates would appear to be beneficial to *T. stoloniferum*, but it is conceivable that there is a life history trade-off between an individual's survival and its reproductive potential (Obeso 2002). If a plant can invest resources in either higher chances of rooted crown persistence or growing more stolons and producing several new ramets, which is the more valuable strategy for individual fitness and/or population persistence? Furthermore, there may be a similar trade-off between sexual and asexual reproduction, in which plants must devote limited energy to either pollination and seed production or the establishment of genetically identical ramets (Franklin 1998); it is likely that environmental contexts affect such a trade-off. For example, Burkhart (2010) found that *T. stoloniferum* in high-light conditions produced more inflorescences. Genetic diversity is very low in BGAD populations (Vincent and Hickey 1996) and presumably clonal reproduction has higher rates of establishment and survival than *T. stoloniferum* starting from seed. Although it is difficult, if not impossible to differentiate clonal plants from those that germinated from seed, it appears that the majority of reproduction is clonal (Vincent and Hickey 1996). While sexual reproduction may have a relatively small effect on patch abundances, it is the only way to increase genetic diversity and disperse *T. stoloniferum* far enough to establish new patches, which could be extremely important to long-term population viability (Franklin 1998). During the course of this study, the numbers of *T. stoloniferum* inflorescences on the BGAD increased dramatically, with both 2013 and 2014 producing more than any other year since regular surveys began in 2001. Researching seed germination rates in the wild and creating a demographic matrix model for *T. stoloniferum* populations would be very helpful in clarifying life history details and prioritizing goals for management strategies.

This study investigates *T. stoloniferum* responses during the two years immediately following treatment application, but effects may become more apparent over a greater length of time. Some of the patch responses had no (or even contradictory) changes over single years, but showed a clearer pattern over the course of all three sampling periods. For example, mowed patches had the greatest abundance increase in 2012-2013, followed by the lowest (non-significant) abundance change in 2013-2014 (Figure 6). The multi-year view of patch responses may be more reliable than that of a single year, but even longer monitoring and continuing treatment applications could reveal accumulative responses that more clearly demonstrate the efficacy of particular treatments. Madarish et al. (2002) found that it took two years after a disturbance event for *T. stoloniferum* densities to increase. Also worth considering is that *T. stoloniferum* in the BGAD appears to rise and fall cyclically, with this study taking place during a period of abundant growth (Dart-Padover et al., in press). It's possible that widespread increases in the population may have masked the effectiveness of the treatments and differences between treatment groups are more evident in years when the population is in decline. Hopefully, future surveys of the BGAD's *T. stoloniferum* population will reveal longer-term responses that could not be observed following only two years of treatment.

The treatments tested in this study are not the only methods that can be used to enhance *T. stoloniferum* populations on the BGAD. *Trifolium stoloniferum* tends to occur where there is increased microtopographic heterogeneity and soil disturbance (Watt 2011). Soil-disturbing methods such as raking or tilling may benefit *T. stoloniferum* populations (although trampling by humans was found to be ineffective, White et al. 1999). Active canopy management may be important in sustaining the canopy gaps and filtered light that *T. stoloniferum* appears to require (Cusick 1989, Madarish and Schuler 2002). Herbicides in addition to the one used in this study (Fusilade II) may be worth investigating. For example, Butyrac 200 (Albaugh, LLC, Ankeny, Iowa) kills most broadleaf plants but is safe for legumes and presumably *T. stoloniferum*. Using Butyrac 200 in conjunction with a grass-specific herbicide could possibly kill all plant competitors in a *T. stoloniferum* patch. Modifying the timing and frequency of artificial disturbance regimes may be more effective. In this study I treated *T. stoloniferum* patches once a year (in late June); applying treatments at various times of the year could have a greater impact on understory vegetation. For example, Becus and Klein (2002) found that mowing *T. stoloniferum* twice before flowering (April-May) and once after flowering (June) was effective at reducing competition. It is not clear whether a treatment needs to be repeated annually to promote growth or if a single disturbance event will have an effect in subsequent years. There may also be some benefit in alternating treatments controlling for grasses in odd years and broadleaf competitors in even years, for example.

This study was intended to assist with the recovery of *T. stoloniferum* on the BGAD by comparing several potential management methods. Using the combination of a grass-specific herbicide and mowing was most effective at enhancing *T. stoloniferum* abundance and survival within two years, but neither method alone was consistently beneficial. Land managers should attempt to address both graminoid and broadleaf plant competition in order to best support *T. stoloniferum* populations. The benefits of using cattle as a *T. stoloniferum* management tool are less clear, and in some cases traditional grazing may be detrimental to the plant's survival and sexual reproduction. However, high-intensity seasonal grazing holds promise and should be investigated further. More replication, longer-term monitoring, and further experimentation with timing will help establish the best practices in conservation of this endangered species, but this study offers a valuable starting point to stewards of *T. stoloniferum* populations across the species' range.

APPENDIX A

TABLES

Table 1. Number of *T. stoloniferum* patches assigned to each of the treatment and grazing groups on the Blue Grass Army Depot (KY). Patches were separated by size (number of ramets) at onset of experiment in 2012. All patches with more than 120 ramets served as controls.

		Treatments				
Grazing Status	**Patch Size**	**Mow**	**Herbicide**	**Mow + Herbicide**	**Control**	**Grand Total**
grazed	1-20	3	2	1	1	**13**
	21-120	1	1	1	1	
	> 120	0	0	0	2	
grazed (enclosure)	1-20	0	4	2	3	**18**
	21-120	3	1	2	2	
	> 120	0	0	0	1	
not grazed	1-20	4	3	4	1	**28**
	21-120	4	4	2	0	
	> 120	0	0	0	6	
Grand Total		**15**	**15**	**12**	**17**	**59**

Table 2. Statistical values for each analysis of *Trifolium stoloniferum* responses on the Blue Grass Army Depot (during all time periods). Bolded lines indicate statistical significance (p < 0.05).

Response Variable	Independent Variable	Years of comparison	Degrees of Freedom	F value / Wald Chi-Square	P value	Multiple Comparison Details
% change in abundance	Treatment	**2012-2014**	**3, 43**	**11.503**	**< 0.001**	**mow+herbicide > herbicide (p = 0.018), control (p = 0.002)**
		2012-2013	**3, 44**	**5.317**	**0.003**	**mow > control (p = 0.009)**
		2013-2014	**3, 45**	**4.685**	**0.006**	**mow+herbicide > mow (p = 0.027)**
	Grazing	2012-2014	2,43	0.350	0.707	
		2012-2013	2, 44	0.450	0.584	
		2013-2014	2, 45	0.242	0.786	
	Interaction	**2012-2014**	**6, 43**	**6.708**	**< 0.001**	**open grazed with mow+herbicide > all other combinations of treatment and grazing**
		2012-2013	6, 44	2.026	0.082	
		2013-2014	6, 45	2.232	0.057	
change in inflorescences per plant	Treatment	2012-2014	3, 44	1.043	0.383	
		2012-2013	3, 44	0.953	0.423	
		2013-2014	3, 43	0.996	0.404	
	Grazing	**2012-2014**	**2,44**	**5.866**	**0.006**	**enclosed grazing > open-grazed (p = 0.012), not grazed (p = 0.024)**
		2012-2013	**2, 44**	**3.613**	**0.035**	**enclosed grazing > not grazed (p = 0.041)**
		2013-2014	2, 43	0.136	0.873	
	Interaction	2012-2014	6, 44	0.510	0.797	
		2012-2013	6, 44	1.502	0.200	
		2013-2014	6, 43	1.479	0.207	

Table 2 (continued)

Response Variable	Independent Variable	Years of comparison	Degrees of Freedom	F value / Wald Chi-Square	P value	Multiple Comparison Details
survival rate	Treatment	**2012-2014**	**3, 44**	**3.105**	**0.036**	**multiple comparisons non-significant, but mow+herbicide > than mow, herbicide, control**
		2012-2013	3, 44	0.730	0.539	
		2013-2014	unequal variance			
	Grazing	**2012-2014**	**2,44**	**4.174**	**0.022**	**not grazed > open-grazed (p = 0.011)**
		2012-2013	2, 44	2.670	0.080	
		2013-2014	unequal variance			
	Interaction	2012-2014	6, 44	1.318	0.269	
		2012-2013	6, 44	1.025	0.422	
		2013-2014	unequal variance			
number of stolons per plant	Treatment	**2013**	**3**	**9.028**	**0.029**	**mow, herbicide, mow+herbicide > control**
		2014	3	5.156	0.161	
	Grazing	2013	2	4.995	0.082	
		2014	**2**	**7.128**	**0.028**	**enclosed grazing, not grazed > open-grazed**
combined length of stolons per plant	Treatment	**2013**	**3**	**8.255**	**0.041**	**mow, herbicide > control**
		2014	3	1.837	0.607	
	Grazing	2013	2	2.788	0.248	
		2014	**2**	**8.056**	**0.018**	**enclosed grazing, not grazed > open-grazed**
recruitment rate	Treatment	2012-2013	3, 43	0.087	0.967	
		2013-2014	3, 42	1.670	0.188	
	Grazing	2012-2013	2, 43	0.757	0.475	
		2013-2014	2, 42	0.033	0.922	
	Interaction	**2012-2013**	**6, 43**	**4.043**	**0.003**	**mow+herbicide with enclosed grazing > all other combinations**
		2013-2014	6, 42	0.356	0.902	

APPENDIX B

FIGURES

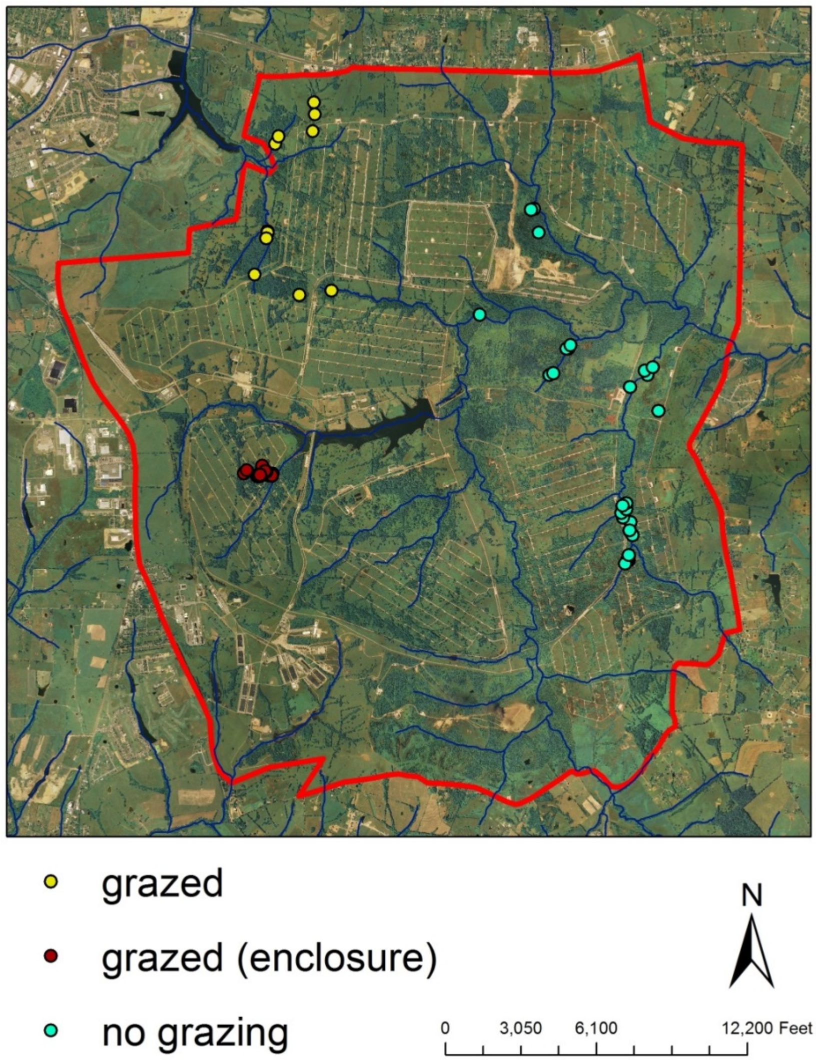

Figure 1. Satellite imagery of the Blue Grass Army Depot, with the outer perimeter outlined in red and streams traced in blue. All *Trifolium stoloniferum* patches that were extant in 2012 are marked, with different colors signifying grazing treatments.

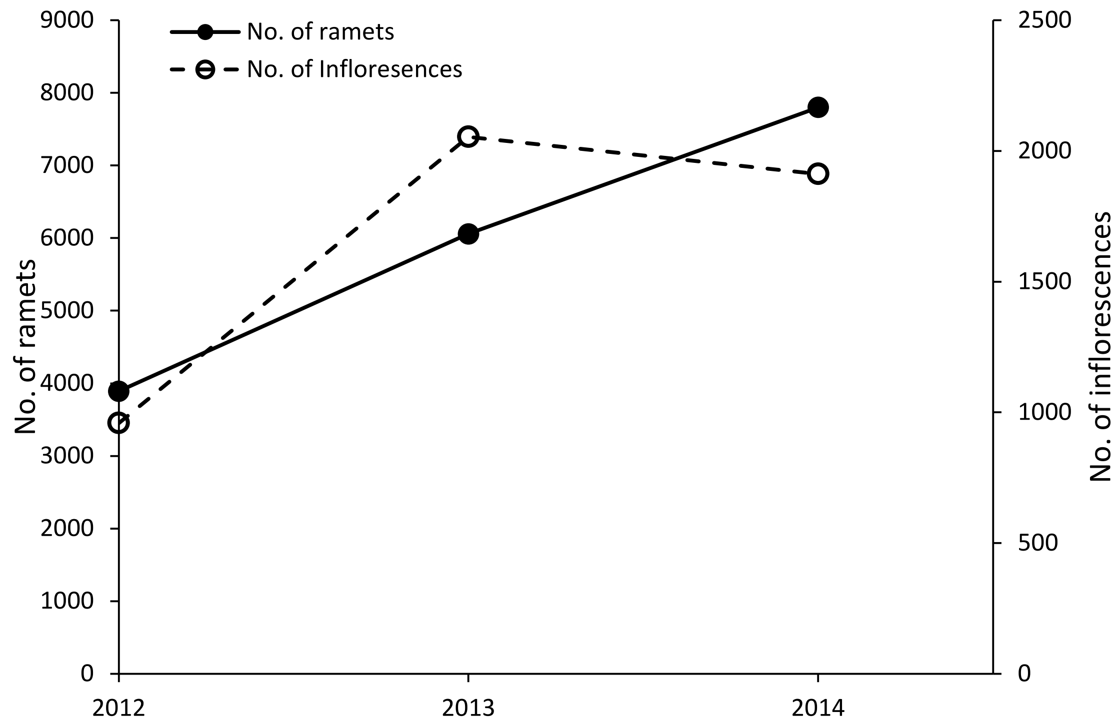

Figure 2. Total numbers of *Trifolium stoloniferum* ramets and inflorescences observed on the Blue Grass Army Depot, 2012-2014.

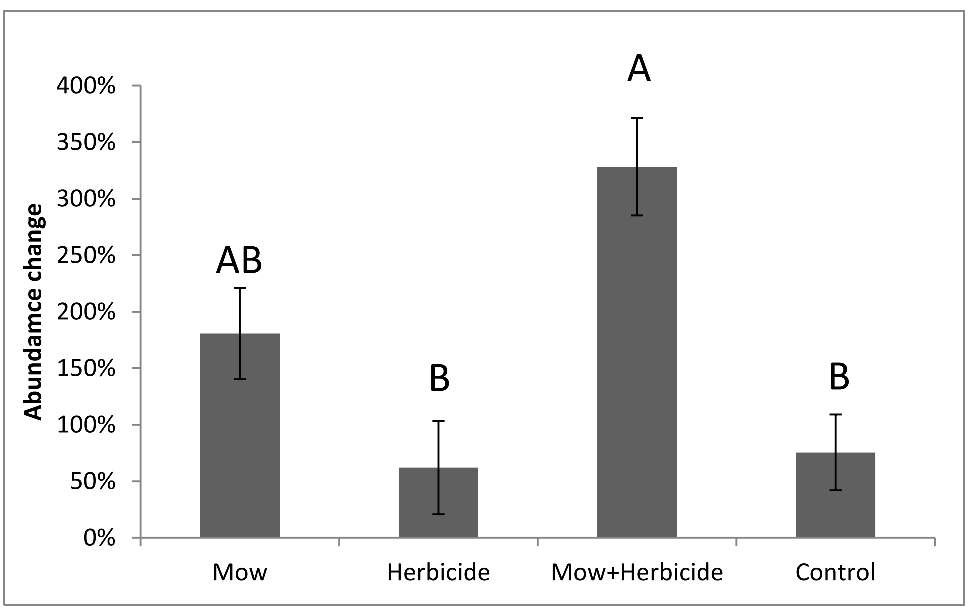

Figure 3. Percent change in abundance (± SE), by treatment, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over two years (2012-2014). Shared letters indicate no significant differences ($p < 0.05$).

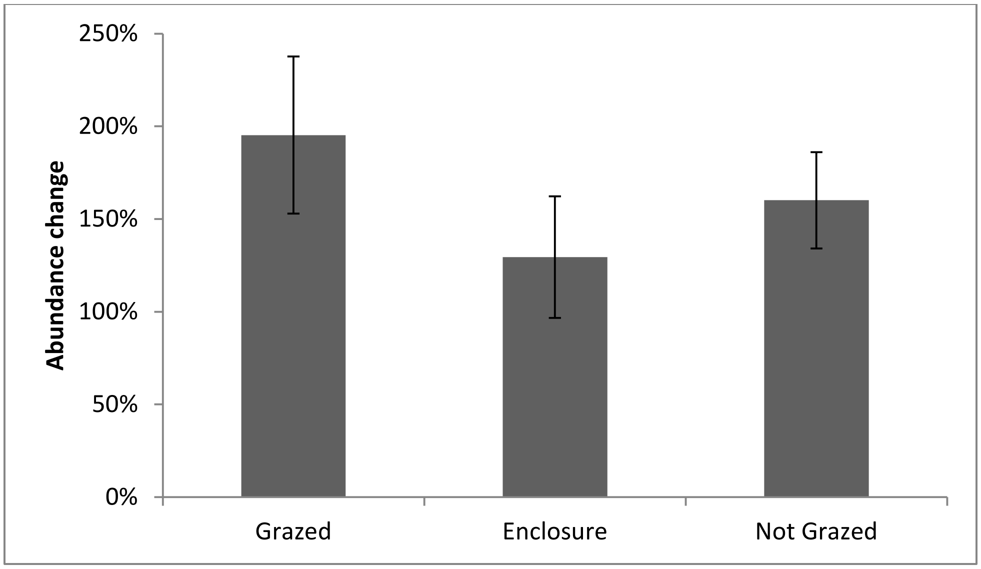

Figure 4. Percent change in abundance (± SE), by grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over two years (2012-2014)

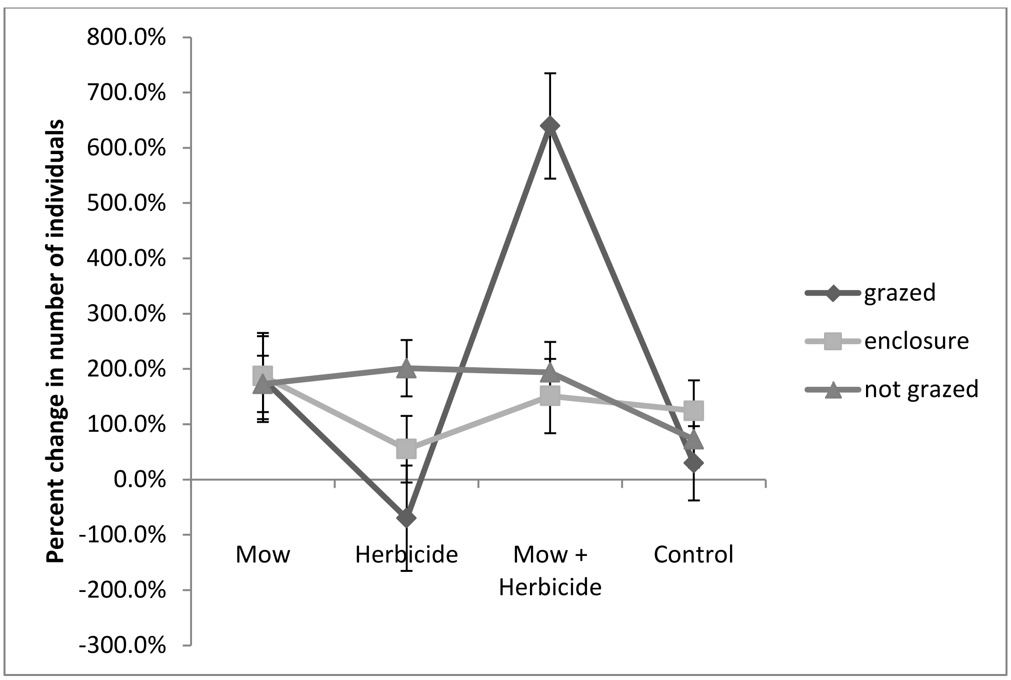

Figure 5. Percent change in abundance (± SE), by treatment and grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over two years (2012-2014).

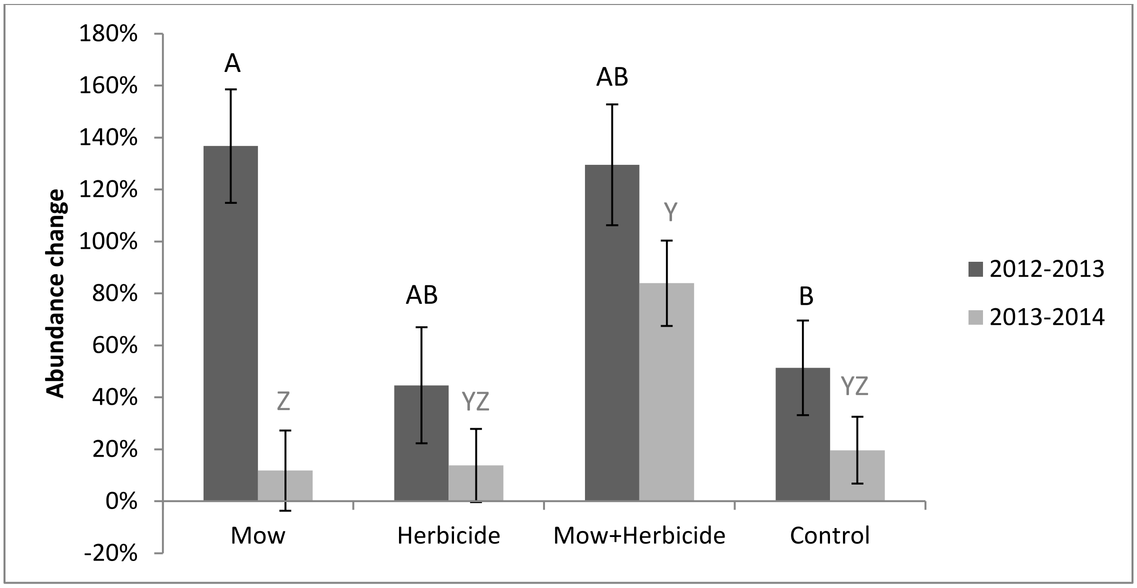

Figure 6. Percent change in abundance (± SE), by treatment, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over single years (2012-2013 and 2013-2014). Shared letters indicate no significant differences among treatments within a single year ($p < 0.05$).

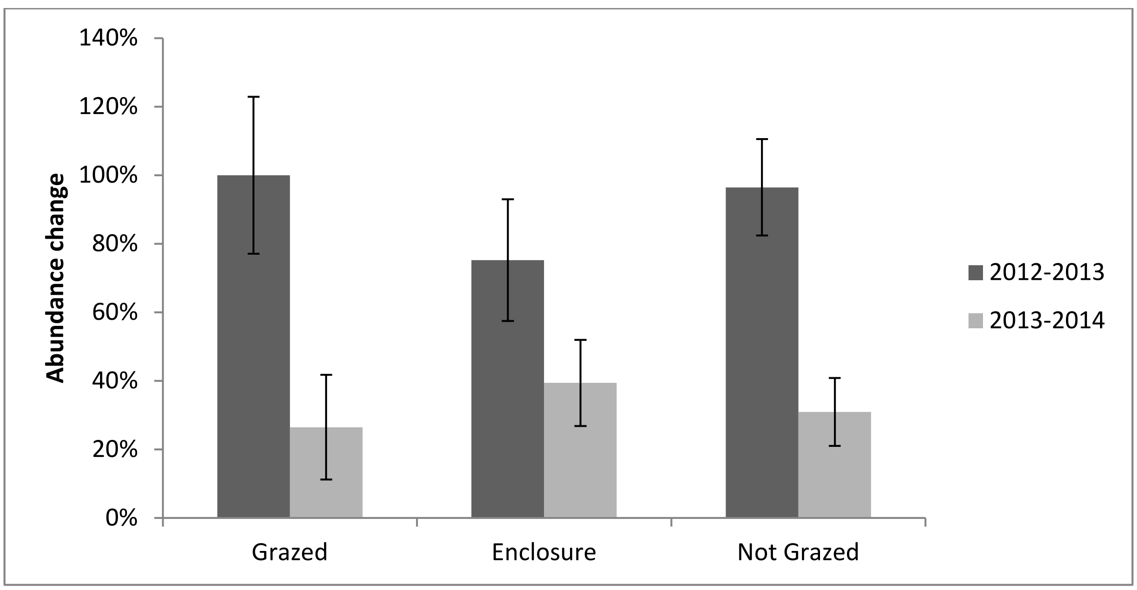

Figure 7. Percent change in abundance (± SE), by grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over single years (2012-2013 and 2013-2014).

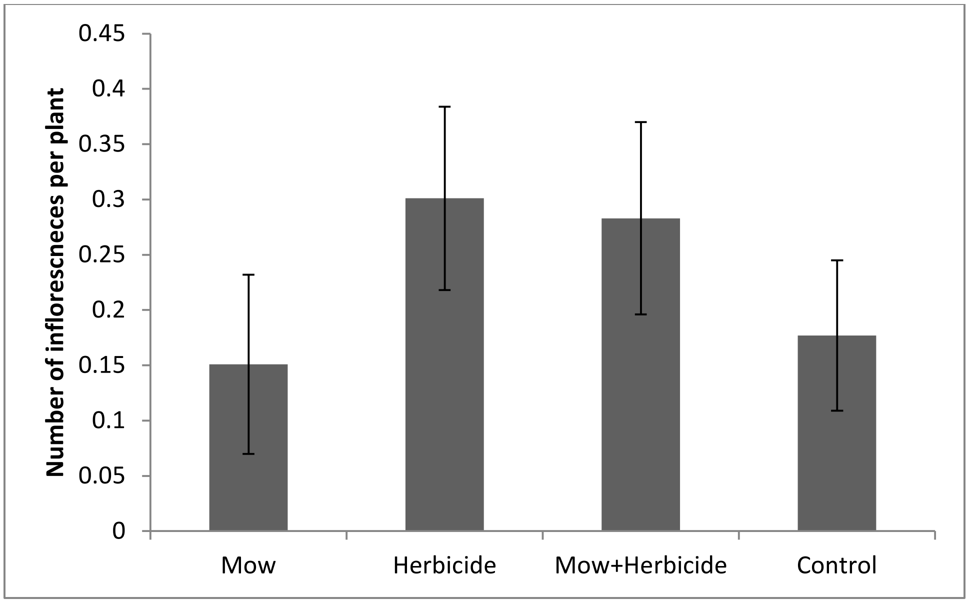

Figure 8. Change in average number of inflorescences per plant (± SE), by treatment, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over two years (2012-2014).

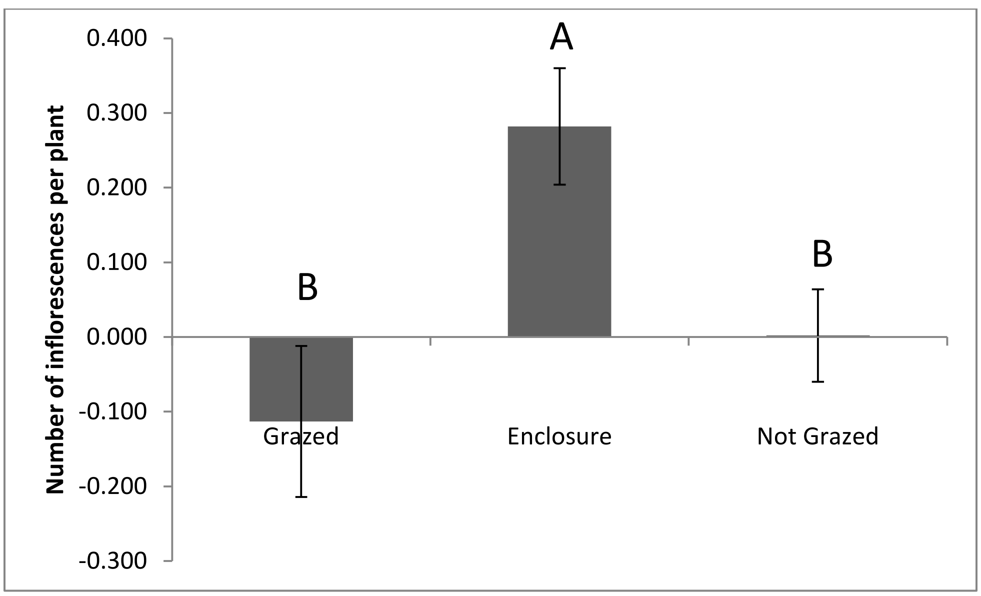

Figure 9. Change in average number of inflorescences per plant (± SE), by grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over two years (2012-2014). Different letters indicate significant differences ($p < 0.05$).

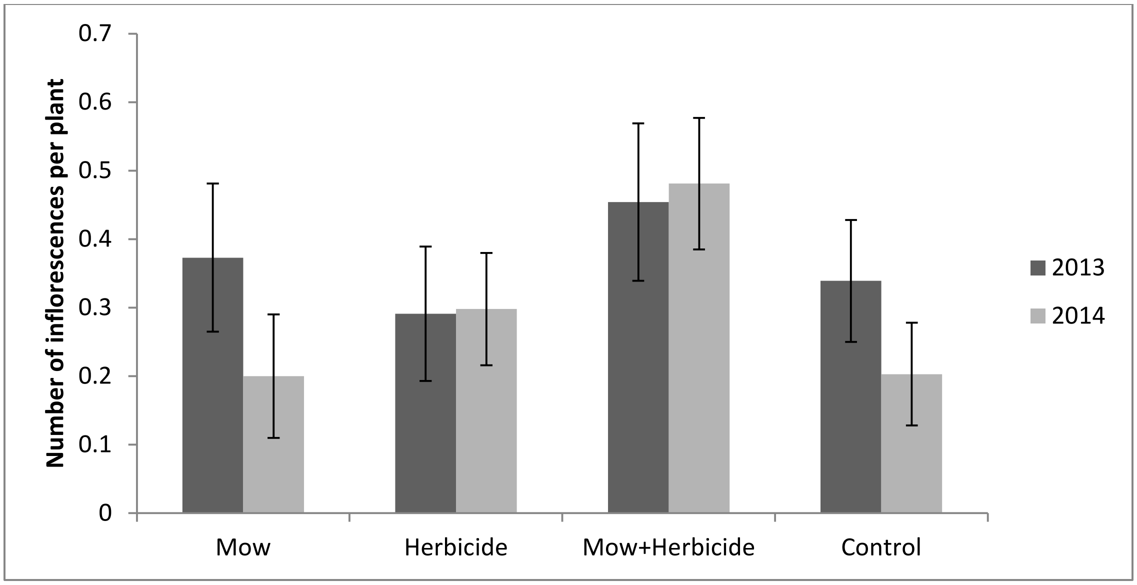

Figure 10. Change in average number of inflorescences per plant (± SE), by treatment, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over single years (2012-2013 and 2013-2014).

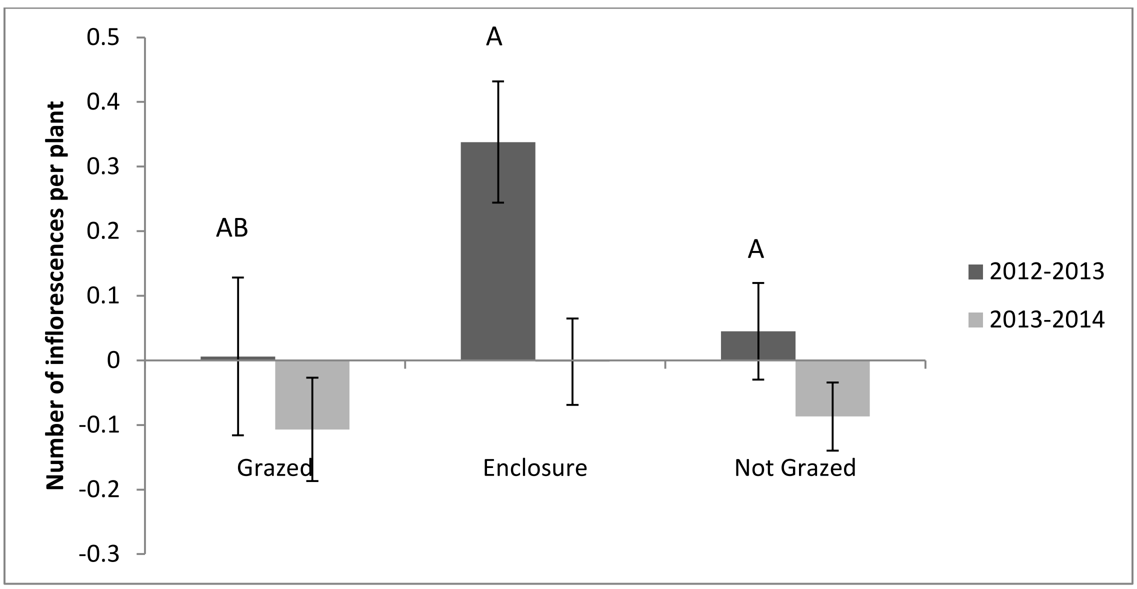

Figure 11. Change in average number of inflorescences per plant (± SE), by grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot over single years (2012-2013 and 2013-2014). Shared letters indicate no significant differences among treatments within a single year ($p < 0.05$). None of the 2013-2014 groups were significantly different from each other.

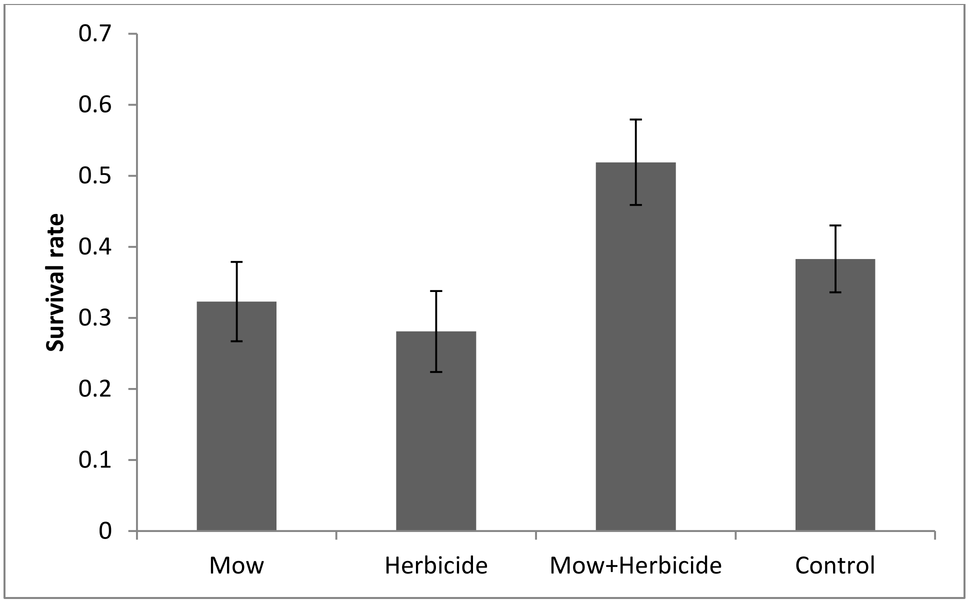

Figure 12. Average survival rate (± SE), by treatment, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot between 2012-2014.

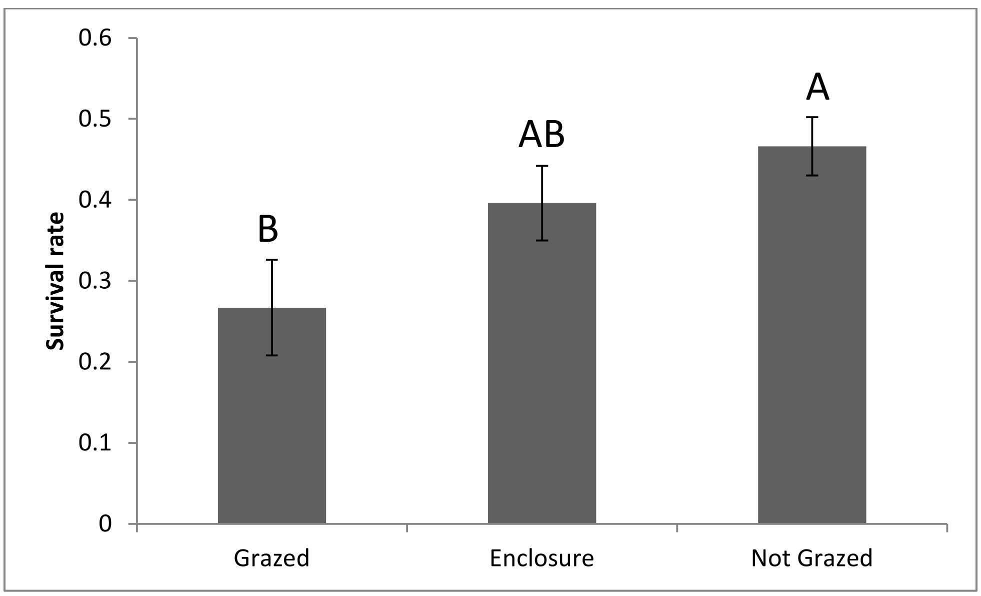

Figure 13. Average survival rate (± SE), by grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot between 2012-2014. Shared letters indicate no significant differences ($p < 0.05$).

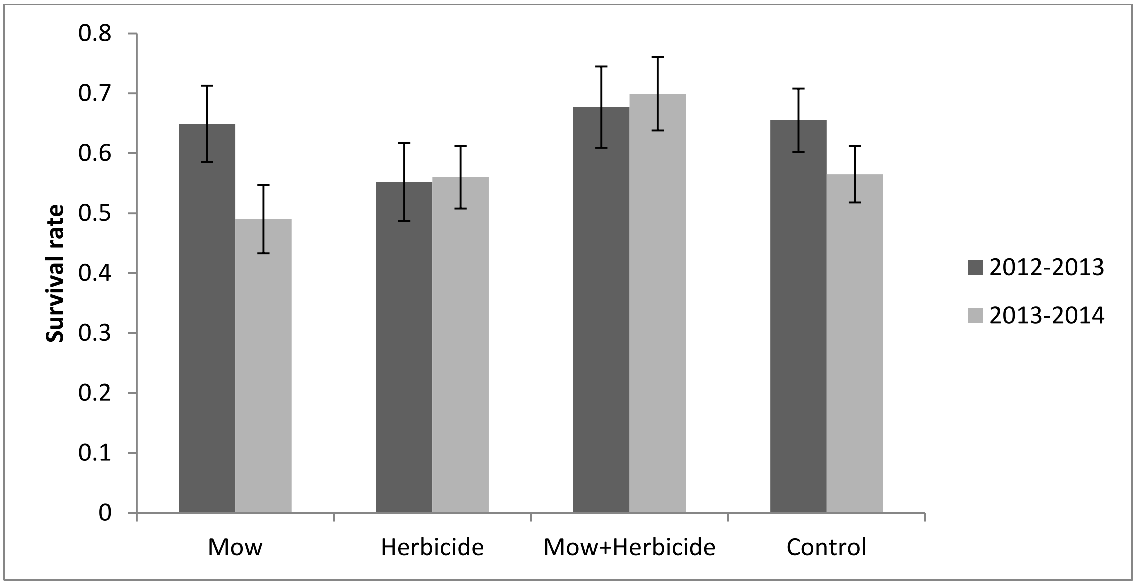

Figure 14. Average survival rate (± SE), by treatment, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot in 2012-2013 and 2013-2014.

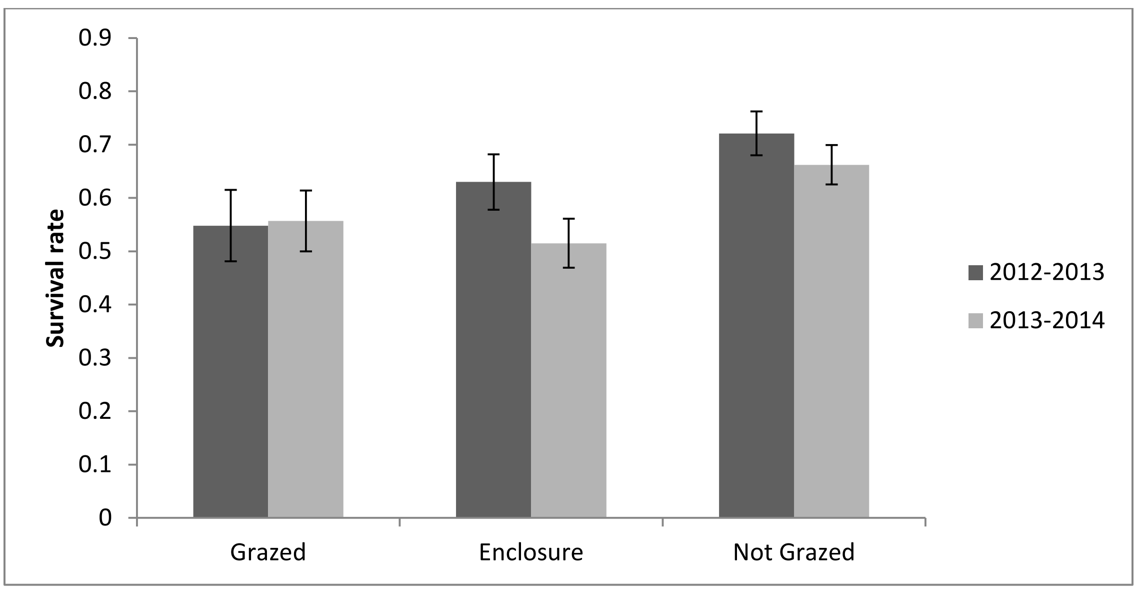

Figure 15. Average survival rate (± SE), by grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot in 2012-2013 and 2013-2014.

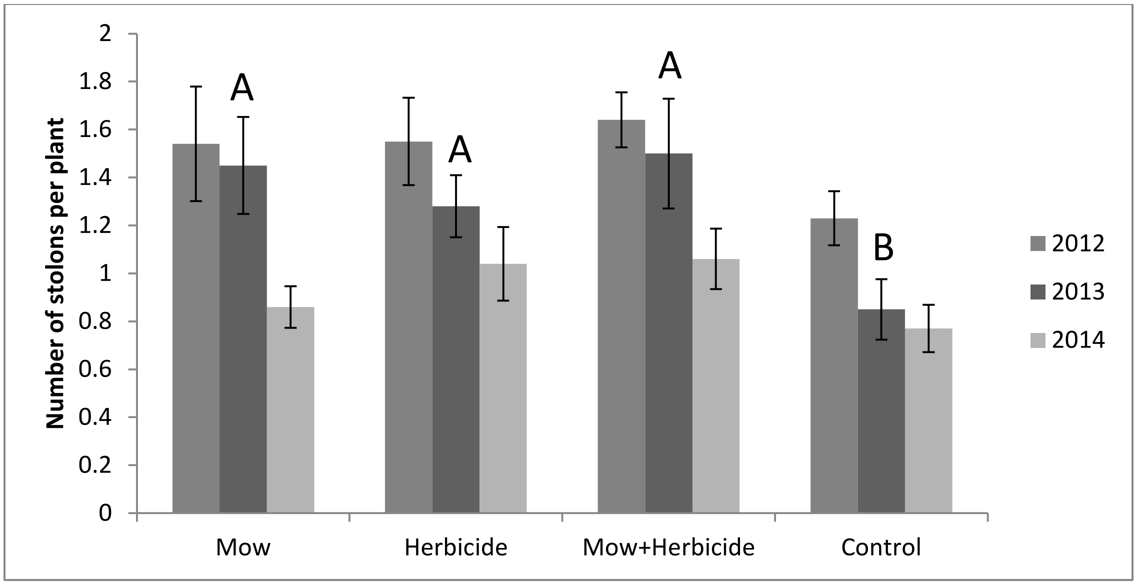

Figure 16. Average number of stolons per plant (± SE), by treatment, for *Trifolium stoloniferum* on the Blue Grass Army Depot in 2012, 2013, and 2014. Shared letters for 2013 indicate no significant differences among treatments within a single year ($p < 0.05$). None of the treatment groups in 2012 or 2014 were significantly different from each other.

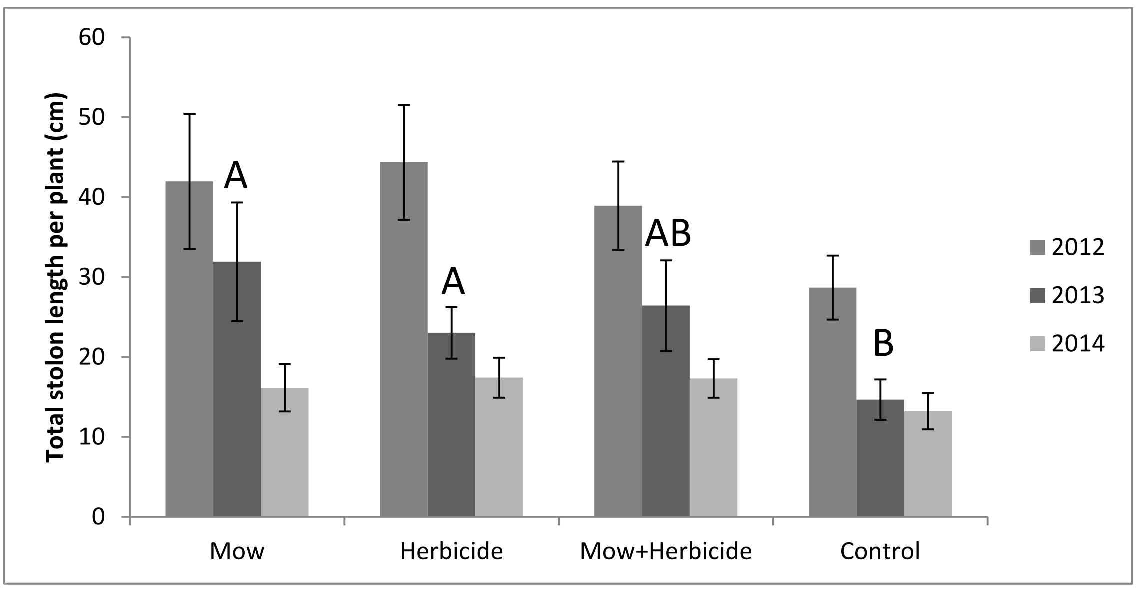

Figure 17. Average combined length of stolons per plant (± SE), by treatment, for *Trifolium stoloniferum* on the Blue Grass Army Depot in 2012, 2013, and 2014. Shared letters for 2013 indicate no significant differences among treatments within a single year ($p < 0.05$). None of the treatment groups in 2012 or 2014 were significantly different from each other.

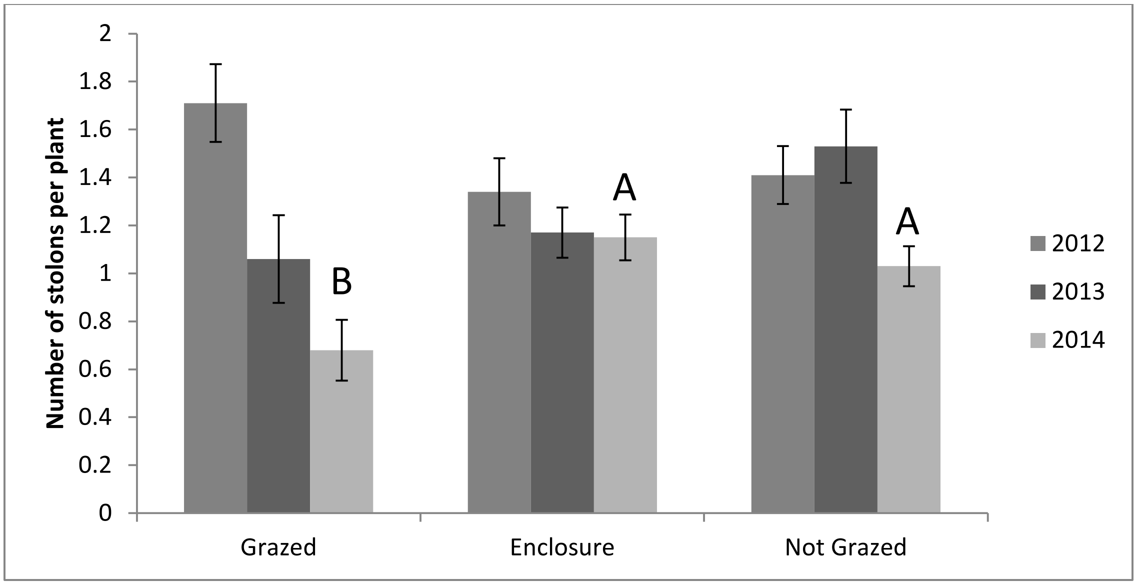

Figure 18. Average number of stolons per plant (± SE), by grazing status, for *Trifolium stoloniferum* on the Blue Grass Army Depot in 2012, 2013, and 2014. Shared letters for 2014 indicate no significant differences among grazing statuses within a single year ($p < 0.05$). None of the grazing treatment groups in 2012 or 2013 were significantly different from each other.

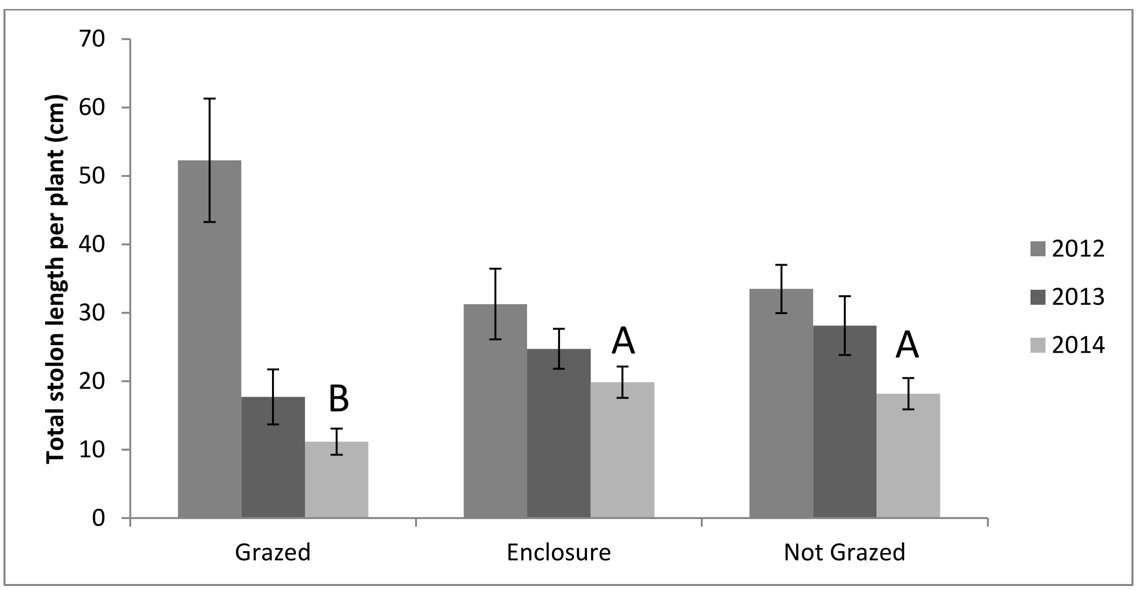

Figure 19. Average combined length of stolons per plant (± SE), by grazing status, for *Trifolium stoloniferum* on the Blue Grass Army Depot in 2012, 2013, and 2014. Shared letters for 2014 indicate no significant differences among grazing statuses within a single year ($p < 0.05$). None of the grazing treatment groups in 2012 or 2013 were significantly different from each other.

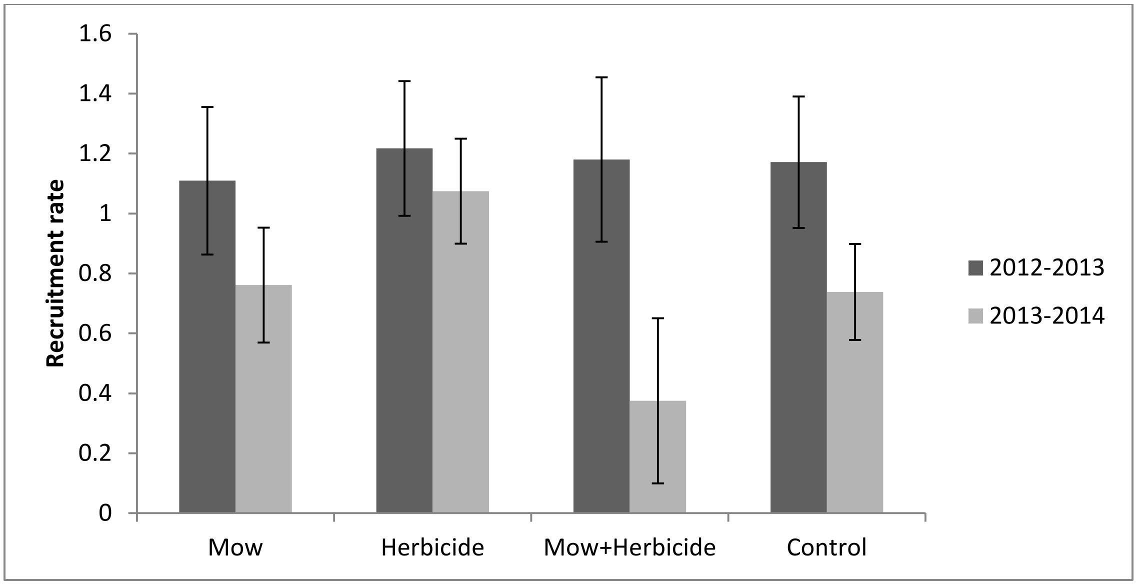

Figure 20. Average recruitment rate (± SE), by treatment, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot in 2012-2013 and 2013-2014.

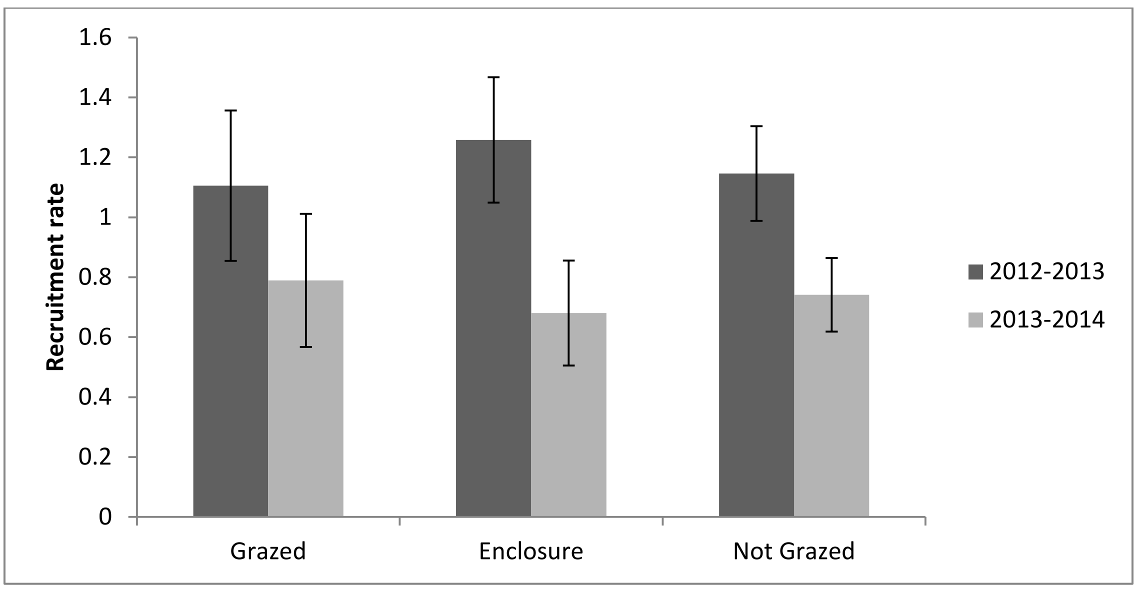

Figure 21. Average recruitment rate (± SE), by grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot in 2012-2013 and 2013-2014.

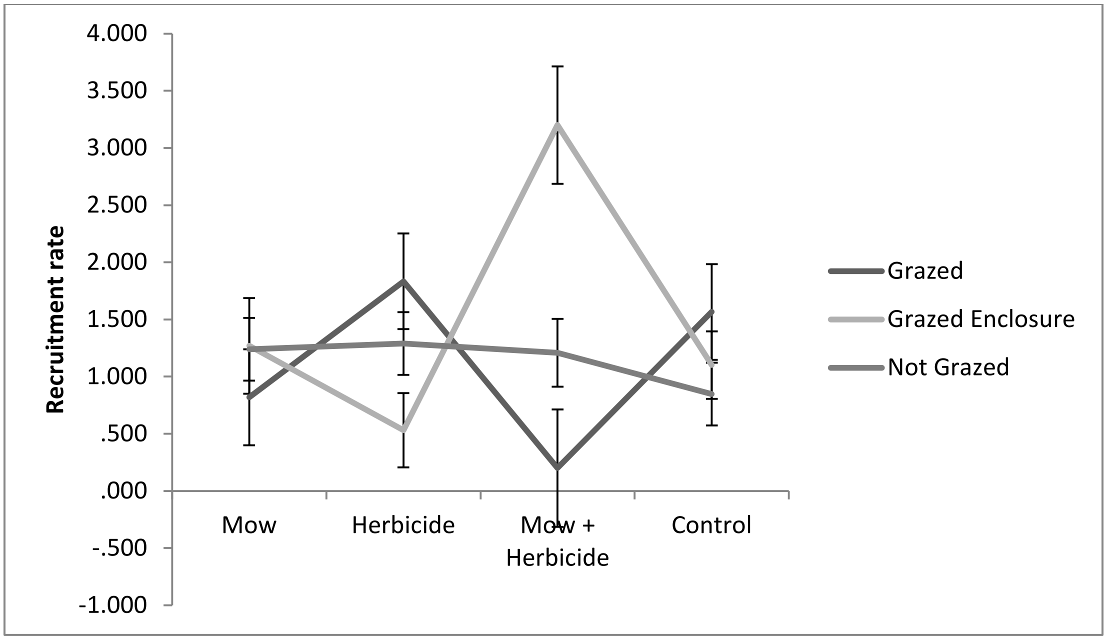

Figure 22. Average recruitment rate (± SE), by treatment and grazing status, for *Trifolium stoloniferum* patches on the Blue Grass Army Depot in 2012-2013.

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